REMARKS

INTRODUCTORY REMARKS

Claims 56, 69-71, 76 and 78 are pending and claims 1-55, 57-68, 72-75 and 77 are canceled.

Applicants would like to thank Examiners Borgeest and Bunner for granting a telephonic interview on March 10, 2009 to discuss the double-patenting rejection.

THE REJECTIONS

Claim rejection under 35 U.S.C. § 103(a)

Claims 56, 70-71, 76 and 78

The Examiner has maintained the rejection of claims 56, 70-71, 76 and 78 under 35 U.S.C. § 103(a) as being obvious over U.S. Patent 6,498,142 ("Sampath") and London et al., Journal of Hypertension, 14:1139-1146 (1996)("London"). The Examiner contends that Sampath teaches methods and pharmaceutical preparations for use in the treatment of mammalian subjects at risk for chronic renal failure comprising administration of OP-1 and other BMPs with appropriate excipients. The Examiner further states that Sampath teaches that subjects indicated for treatment are those at risk for renal failure and that measuring GFR is a method of estimating renal function. Finally, the Examiner contends that Sampath teaches successful attenuation of renal failure in an art-accepted model for renal failure. The Examiner contends that London teaches the administration of ACE inhibitors for treating hypertensive individuals with endstage renal disease with the effect that ACE inhibition resulted in more efficient ventricular-vascular coupling and decreased left ventricular load. Further, the Examiner states that London teaches that ACE inhibition improves

blood pressure in patients with renal disease, a condition often complicated with hypertension. The Examiner contends that although neither Sampath nor London teaches or suggests that the combination of BMP morphogens and ACE inhibitors would lower proteinuria, the claims of the instant application are drawn to a product whose ability to lower protein in the urine is intrinsic to the functioning of this combination. The Examiner also states that Ritz et al., Am. J. Hypertension, 8:535-85 (1995) ("Ritz") and de Zeeuw et al., Canadian Journal of Cardiology, 11(Suppl.F):41F-44F (1995) ("de Zeeuw") provide further evidence that "it was known in the art that ACE inhibition was not only useful for treatment of hypertension but also attenuating renal injury (*e.g.*, proteinuria) in individuals with diabetic nephropathy at risk for renal failure." Finally, the Examiner states that the specification does not define "synergistic" and that applicants "do not outline why the synergy between OP-1 and enalapril would be unexpected, especially given that the prior art teaches both of these agents are useful in the treatment of renal disease." Applicants traverse.

Applicants maintain that nothing in Sampath, London, Ritz and de Zeeuw alone or in combination would lead the skilled worker to the invention recited in the claims of the instant application. The claims of the instant application recite a composition comprising an ACE inhibitor and specific BMP morphogens, wherein the combination of the ACE inhibitor and BMP morphogen is capable of inducing a synergistic effect on reducing proteinuria levels in a diabetic neuropathy model.

By contrast, Sampath discloses that OP-1 treatment of nephrectomized rats resulted in overall improvement of kidney tissue morphology, increased mesangial or perivascular thickening, decreased glomerular sclerosis and loop collapse, decreased presence of "scattered" sclerosis and

microaneurysms and an increase in viable glomeruli. Sampath also discloses that OP-1 treatment of nephrectomized rats resulted in a stabilization of GFR as compared to control. Applicants note that improved GFR is ***not*** synonymous with improved proteinuria. Therefore, contrary to the Examiner's assertion, Sampath does not teach or suggest that OP-1 reduces proteinuria levels. And, nothing in any of London, Ritz or de Zeeuw, alone, or in combination remedies this deficiency.

London discloses that Quinapril treatment of hypertensive patients with endstage renal disease resulted in a blood pressure independent decrease in arterial wave reflections and that the consequence of this was a decrease in pulsatile pressure load in the central arteries with increased aortic distensibility. Nowhere in London is there any teaching or suggestion that ACE inhibitors reduce proteinuria. Ritz and de Zeeuw, however, disclose that ACE inhibitors reduce proteinuria. Notwithstanding, nothing in any of London, Ritz or de Zeeuw teaches or suggests that BMPs improve proteinuria or that ACE inhibitors should be combined with BMPs to produce a ***synergistic*** effect on proteinuria.

Applicants further note that "synergistic" effect as used in the instant application has its plain meaning—*i.e.*, an effect for the combination that is greater than the sum of the effects for the individual agents. This is evidenced by the results disclosed in Example 4 and Figure 27. For example, the application teaches that the combination of OP-1 and enalapril is superior in reducing proteinuria than either agent alone: "OP-1 (10 or 30 μg/kg) or enalapril reduced the proteinuria level, from about 180 mg/dL/24hr to about 80 (or 110) or 140 mg/dL/24hr, respectively. In contrast, the combination of OP-1 and enalapril dramatically reduced the proteinuria level to as low as about 30 mg/dL/24hr (p<0.01)." See, page 143, lines 6-10. This disclosure demonstrates that the

effect of combining OP-1 and enalapril on decreasing proteinuria was more than additive.

Thus, applicants submit that nowhere in any of Sampath, London, Ritz and de Zeeuw is there any teaching or suggestion that BMPs reduce proteinuria. And, nowhere in any of these documents is there any teaching or suggestion to combine a BMP and an ACE inhibitor or that the combined effect on reducing proteinuria would be ***synergistic***. In fact, Ritz discloses that the combination of an ACE inhibitor and a calcium channel antagonist in renal patients resulted only in ***an additive*** effect on reduction of proteinuria. Indeed, based on the teachings of Ritz, to the extent that a combination of ACE inhibitors and other renal agents were taught, the skilled worker would expect no more than an additive effect on proteinuria.

In re Huellmantel, 324 F.2d 998, 139 U.S.P.Q. 496 (CCPA 1963) and *In re Meinhardt*, 392 F.2d 273, 157 U.S.P.Q. 270 (CCPA 1968), which were relied upon by the Examiner to support her contention that synergism is not sufficient to overcome a *prima facie* case of obviousness because such an effect can either be expected or unexpected, are inapposite. In both cases, the prior art taught or suggested synergism. By contrast, here, nothing in the prior art teaches or suggests synergism. As discussed above, Ritz at best discloses an ***additive effect--not*** a ***synergistic effect***. The synergistic results obtained by applicants when they combined a BMP with an ACE inhibitor were unexpected.

More than that, the state of the art at the time of the invention taught away from combining ACE inhibitors with other agents. Indeed, the prior art taught that caution should be exercised when combining ACE inhibitors with other treatment compounds. For example, the combination of an ACE inhibitor and a non-steroidal anti-inflammatory drug has been demonstrated

to be nephrotoxic. *See, e.g.*, Adhiyaman et al., S.R.Soc.Med. 94:512-14 (2001), attached herewith as Appendix A. Similarly, the combination of an ACE inhibitor and cyclosporin has been associated with causing acute renal failure, and the combination of an ACE inhibitor with probenicid has been linked to the augmentation of renal excretion of urate, which may ultimately result in gouty stones. *See, e.g.*, Murray et al., American Journal Kidney Diseases. 16: 66-69 (1990), attached herewith as Appendix B and Leary et al., and Cardiovascular Drugs and Therapy. 1:29-38 (1987), attached herewith as Appendix C.

Therefore, based on the state of the art, the skilled worker would not have been motivated to combine an ACE inhibitor with a BMP to reduce proteinuria. Nor would the skilled worker have expected that the combination would result in a synergistic effect.

For all of the above reasons, applicants submit that the claims of the instant application are not obvious and request that the Examiner withdraw this rejection.

Claims 69

The Examiner has maintained the rejection of claim 69 under 35 U.S.C. § 103(a) as being obvious over Sampath, London and Salvetti et al., Drugs, 40:800-28 (1990) ("Salvetti"). The Examiner states that Sampath and London do not teach that the ACE inhibitor is enalapril but that Salvetti reviews and compares ACE inhibitors including enalapril. The Examiner further states that Salvetti teaches that enalapril is more potent and has a longer duration of action. The Examiner concludes that the skilled worker would be motivated to use enalapril because of the greater potency and duration and could have reasonably expected success. Applicants traverse.

As discussed above, nothing in Sampath or London, either alone or in combination, teaches or suggests combining an ACE inhibitor and a BMP for reducing proteinuria levels or that the combination would have a synergistic effect (*i.e.*, greater than additive) on reducing proteinuria levels. Salvetti does not remedy this deficiency. Salvetti discloses ACE inhibitors and that enalapril is more potent than other ACE inhibitors and has a longer duration of action. Salvetti does not teach or suggest that BMPs improve proteinuria, combining enalapril or any other ACE inhibitor with BMPs, or that the combination would have a synergistic effect on reducing proteinuria. Accordingly, applicants respectfully request that the Examiner withdraw this rejection.

Obviousness-Type Double Patenting

U.S. Patent No. 6,677,432, London and Vukicevic
Claims 56, 71, 76 and 78

The Examiner has maintained the rejection of claims 56, 71, 76 and 78 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-13 of U.S. Patent No. 6,677,432 ("the '432 patent") in view of London and further in view of Vukicevic et al., J. Clin. Invest., 102(1):202-214, (1998) ("Vukicevic"). Applicants traverse.

Applicants maintain that claims 1-13 of the '432 patent cannot form the basis for an obviousness-type double patenting rejection. The claims of the instant application recite a pharmaceutical composition comprising an ACE inhibitor and a BMP having its ***native sequence***. By contrast, the claims of the '432 patent recite ***OP-1 mutants*** having amino acid substitutions at at least one residue selected from residues 400, 402, 421, 422, 426 and 431. The OP-1 mutants recited

in the '432 patent have, *inter alia*, altered refolding properties relative to naturally occurring proteins and can have altered stability, specific activity, solubility, bioactivity, and/or biospecificity attributes. *See, e.g.*, the '432 patent, column 4, lines 35-40. Therefore, the OP-1 mutants recited in the '432 patent are structurally and functionally different from the BMP proteins recited in the claims of the instant application.

The Examiner's contention that Vukicevic recites OP-1 does not carry the day. The comparison must be between the claims of the '432 patent and the claims of the instant application. As discussed above, the claims of the '432 patent recite OP-1 ***mutants*** and the claims of the instant application do not. Moreover, Vukicevic does not teach or suggest that OP-1 has any effect on reducing proteinuria. While Vukicevic demonstrates that OP-1 treatment reduces serum creatinine and blood urea nitrogen in a rat model of ischemic acute renal failure, Vukicevic does ***not*** provide any evidence that OP-1 treatment has any effect on reducing proteinuria levels.

The alleviation of one symptom of kidney disease, as demonstrated by the reduction of serum creatinine and blood urea nitrogen levels in Vukicevic, would not lead the skilled worker to believe that that same treatment would be effective in alleviating ***all*** symptoms of kidney disease. Indeed, both Ritz and de Zeeuw teach that antihypertensive treatments, other than ACE inhibitors, were used originally only for reducing elevated systemic blood pressure associated with exacerbating kidney disease. ACE inhibitors were considered distinct from other antihypertensive treatments because they possessed the additional unexpected effect of reducing proteinuria. Similarly, applicants are the first to show that a BMP has the unexpected effect of reducing proteinuria in a diabetic nephropathy model.

More than that, applicants have demonstrated that the combination of a BMP and an ACE inhibitor unexpectedly results in synergistic reduction of proteinuria levels in a diabetic nephropathy model. Nothing in the combination of the '432 patent claims, Vukicevic and London teaches or suggests that the combination of a BMP and an ACE inhibitor would have a synergistic effect on reducing proteinuria levels. Indeed, as discussed above the prior art teaches that the combination of ACE inhibitors with other agents leads to undesirable effects. At best, based on the teachings of Ritz, the skilled worker would expect that the combination of an ACE inhibitor with a BMP would result in an additive effect.

For all of the above reasons, the pending claims of the instant application are ***patentably distinct*** and do not define an invention that is merely an obvious variation of the invention of claims 1-13 of the '432 patent.

U.S. Patent No. 6,846,906, London and Vukicevic
Claims 56, 71, 76 and 78

The Examiner has rejected claims 56, 71, 76 and 78 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-5 of U.S. Patent No. 6,846,906 ("the '906 patent") in view of London and further in view of Vukicevic. Applicants traverse.

Applicants maintain that claims 1-5 of the '906 patent cannot form the basis for an obviousness-type double patenting rejection for the same reasons that claims 1-13 of the '432 patent cannot form the basis for an obviousness-type double patenting rejection.

Claims 1-5 of the '906 patent are directed to ***chimeric TGF-β superfamily protein***s, wherein the finger 2 subdomain is from CDMP-2 and the finger 1 and heel subdomains are from a different member of the superfamily. By contrast, the claims of the instant application recite a pharmaceutical composition comprising an ACE inhibitor and a BMP having its ***native sequence***. The chimeric proteins recited in the '906 patent have, *inter alia*, altered refolding properties relative to naturally occurring proteins and can have altered stability, surface binding, solubility, bioactivity, and/or biospecificity attributes. See, *e.g.*, the '906 patent, column 4, lines 40-45. Therefore, the OP-1 chimeric proteins recited in the '906 patent are structurally and functionally different from the BMP proteins recited in the claims in the instant application.

As in the case of the '432 patent, the Examiner's position that Vukicevic recites OP-1 is not relevant. The claims of the '906 patent do not. In addition, as discussed above, Vukicevic does not teach that OP-1 has any effect on reducing proteinuria levels.

Further, as discussed above, applicants have demonstrated that the combination of a BMP and an ACE inhibitor synergistically reduced proteinuria levels in a diabetic nephropathy model. Nothing in the claims of the '906 patent together with London and/or Vukicevic teaches or suggests this synergistic effect. And, the prior art suggests no more than an additive effect. Therefore, the skilled worker would not have had a reasonable expectation that the combination of a BMP and an ACE inhibitor would have a synergistic effect on reducing proteinuria levels. Therefore, the pending claims of the instant application are patentably distinct from claims 1-5 of the '906 patent. Accordingly, applicants request that the Examiner withdraw this obviousness-type double patenting rejection.

U.S. Application No. 10/816,768, London and Vukicevic
Claims 56, 71, 76 and 78

The Examiner has maintained the provisional rejection of claims 56, 71, 76 and 78 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 10 and 16-18 of co-pending U.S. Application No. 10/816,768 ("the '768 application") in view of London and further in view of Vukicevic. The Examiner contends that claims 10 and 16-18 of the '768 application recite the same BMP morphogens as claim 56. Applicants traverse.

Applicants maintain that claims 10 and 16-18[2] of the '768 application cannot form the basis for an obviousness-type double patenting rejection for the same reasons that claims 1-13 of the '432 patent and claims 1-5 of the '906 patent cannot form the basis for an obviousness-type double patenting rejection.

Contrary to the Examiner's assertion, claims 10 and 16-18 do not recite the same BMP morphogens as claim 56, 71, 76 and 78 of the instant application. Claims 10 and 16 of the '768 application are directed to TGF-β family ***fusion protein*** having a leader sequence domain operatively linked to the C-terminal domain of the TGF-β family member protein. Claims 17 and 18 of the '768 application are directed to a ***heterodimer*** comprising a first subunit being a TGF-β ***fusion protein*** and a second subunit that is either a TGF-β fusion protein or a wild type protein.

Contrary to the Examiner's assertion, claims 10 and 16-18 of the '768 application do not recite the same BMP morphogens as the claims of the instant application. The pending claims

[2] Applicants note that claims 10 and 16-18 of the '768 application are currently withdrawn.

of the instant application recite specific BMPs having their native sequence, not TGF-β family fusion proteins.

Further, applicants have demonstrated that the combination of a BMP and an ACE inhibitor synergistically reduces proteinuria levels. Nothing in the combination of claims of the '768 application, London or Vukicevic teaches or suggests a synergistic effect on reducing proteinuria levels. Indeed, nothing in the prior art suggests a synergetic effect. The art teaches either deleterious effects or at best an additive effect. Thus, the skilled worker would have no reasonable expectation that the combination of a BMP and an ACE inhibitor would have a synergistic effect, or any effect, on reducing proteinuria levels. Therefore, the pending claims of the instant application are patentably distinct from claims 10 and 16-18 of the '768 application. Accordingly, applicants request that the Examiner withdraw this provisional obviousness-type double patenting rejection.

U.S. Patent No. 6,677,432, London, Vukicevic and Salvetti
Claims 56 and 69

The Examiner has maintained the rejection of claims 56 and 69 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-13 of the '432 patent in view of London and further in view of Vukicevic and Salvetti. Applicants traverse.

As discussed above, claims 1-13 of the '432 patent, either alone or in combination with London and/or Vukicevic, cannot form the basis for an obviousness-type double patenting

rejection. Salvetti does not remedy this deficiency. Salvetti discloses ACE inhibitors and that enalapril is more potent than other ACE inhibitors and has a longer duration of action.

Claims 1-13 of the '432 patent, either alone or in combination with London, Vukicevic and/or Salvetti do not teach or suggest that the combination of an ACE inhibitor with the specifically recited BMPs would have a synergistic effect, or any effect, on reducing proteinuria levels, as recited in the pending claims of the instant application. Such a synergistic effect was unexpected.

For the same reasons discussed above for claims 56, 71, 76 and 78, applicants request that the Examiner withdraw this obviousness-type double patenting rejection.

U.S. Patent No. 6,846,906, London, Vukicevic and Salvetti
Claims 56 and 69

The Examiner has maintained the rejection of claims 56 and 69 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-5 of the '906 patent in view of London and further in view of Vukicevic and Salvetti.

As discussed above, claims 1-5 of the '906 patent, either alone or in combination with London and/or Vukicevic, cannot form the basis for an obviousness-type double patenting rejection. And, Salvetti does not remedy this deficiency.

Claims 1-5 of the '906 patent, either alone or in combination with London, Vukicevic and/or Salvetti do not teach or suggest that the combination of an ACE inhibitor with the specifically recited BMPs would have a synergistic effect, or any effect, on reducing proteinuria

levels, as recited in the pending claims of the instant application. The synergistic effect was unexpected.

For the same reasons discussed above for claims 56, 71, 76 and 78, applicants request that the Examiner withdraw this obviousness-type double patenting rejection.

U.S. Application No. 10/816,768, London, Vukicevic and Salvetti
Claims 56 and 69

The Examiner has maintained the provisional rejection of claims 56 and 69 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 10 and 16-18 of the '768 application in view of London and further in view of Vukicevic and Salvetti.

As discussed above, claims 10 and 16-18[3] of the '768 application, either alone or in combination with London and/or Vukicevic, cannot form the basis for an obviousness-type double patenting rejection. Salvetti does not remedy this deficiency.

Claims 10 and 16-18 of the '768 application, either alone or in combination with London, Vukicevic and/or Salvetti do not teach or suggest that the combination of an ACE inhibitor with the specifically recited BMPs would have a synergistic effect, or any effect, on reducing proteinuria levels, as recited in the pending claims of the instant application. Such a synergistic effect was unexpected.

For the same reasons discussed above for claims 56, 71, 76 and 78, applicants request that the Examiner withdraw this provisional obviousness-type double patenting rejection.

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[3] Applicants note that claims 10 and 16-18 of the '768 application are currently withdrawn.

CONCLUSION

In view of the foregoing remarks, applicants request that the Examiner reconsider and withdraw all outstanding rejections and allow the pending claims.

The Examiner is invited to telephone applicants' representatives regarding any matter that may be handled by telephone to expedite allowance of the pending claims.

Respectfully submitted,

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APPENDIX A

PRACTICE

Nephrotoxicity in the elderly due to co-prescription of angiotensin converting enzyme inhibitors and nonsteroidal anti-inflammatory drugs

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J R Soc Med 2001;94:512–514

SUMMARY

Both angiotensin converting enzyme inhibitors and nonsteroidal anti-inflammatory drugs can lead to functional renal insufficiency. In an observational study we assessed the frequency of this adverse effect in patients aged over 75 years receiving these drugs in combination. In one year, out of 1500 patients whose records were screened, 12 were prescribed this combination. 2 developed acute renal failure, of whom one died and the other recovered after discontinuation of both drugs. 4 patients showed deterioration in renal function, which returned to normal after one of the drugs was stopped. Renal function remained stable in 6 patients: patients with deterioration in renal function were older and more likely to be on diuretics.

This drug combination is commonly nephrotoxic in the elderly and should be avoided, especially in those taking diuretics.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors are increasingly used in the elderly for heart failure and hypertension. Another group of drugs commonly used in this age group are the nonsteroidal anti-inflammatories (NSAIDs). Since cardiac failure and hypertension often coincide with chronic pain in the elderly, co-prescription of these drugs is not infrequent. However, data on the renal safety of this combination in the elderly are very limited. A study of trandalopril and indometacin in hypertensive patients did not show any change in renal functional reserve, but the patients were young (mean age 53.5 years) and did not have any co-morbidity[1]. We undertook this study to assess the effects of combined therapy on renal function in the elderly.

METHODS

A prospective observational study was conducted in a district general hospital over one year. Patients above 75 years of age from the care-of-the-elderly unit formed the study group. We reviewed all completed discharge prescriptions during this period. Patients with normal renal function on long-term treatment with either an ACE inhibitor or a NSAID (for at least three months), in whom the other drug was added during the present admission, were identified. During the study period, consultants in charge were unaware of the inquiry and so patients were prescribed this combination because of their perceived need, not to recruit them into the investigation. We noted baseline renal function at the start of combined therapy and again when the patients came for their routine outpatient review.

For both occasions we recorded use of other medications, especially diuretics; and if, on review, any drug had been stopped, the reason was identified from the case notes. In patients who showed renal deterioration, any associated illnesses and change in medication were noted. Acute renal failure (ARF) was defined as a sudden increase in serum creatinine from normal to more than 180 μmol/L[2]. Since there is no existing definition for renal deterioration, we arbitrarily defined it as an increase in serum creatinine of 50% above the baseline within six weeks (six weeks being chosen because it is the usual interval for an outpatient review after discharge). Even though this was an observational study, when patients failed to turn up for their review we requested the general practitioner to do the blood tests in the best interests of the patient.

RESULTS

During the one-year study period there were 2050 admissions to the unit. After exclusion of those with

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incomplete prescriptions, those transferred to community/rehabilitation hospitals (for which discharge prescriptions were usually unavailable), and those who died, information was collected on 1500 patients. Of these, 12 (6 males) had received an ACE inhibitor and a NSAID in combination. In 6 the ACE inhibitor had been prescribed first, in 6 the NSAID.

2 patients developed ARF, at six weeks and three months. Both were on diuretics and had had the NSAID added to ACE inhibitor. In the first patient, renal function did not improve after both drugs were stopped. Renal support was not considered because of coexisting metastatic liver disease and the patient died. The second patient had diarrhoea and hyponatraemia before the development of ARF. Both drugs were discontinued and renal function returned to normal after volume repletion.

4 patients showed deterioration in renal function between eight and twelve weeks. All were on diuretics and the dosage had not been altered since discharge. The NSAID was stopped in 3 and the ACE inhibitor in one, whereupon renal function returned to normal. In this group NSAID had been added to ACE inhibitor in one and ACE inhibitor to NSAID in 3. In the remaining 6 patients, renal function remained unaffected at six months: 2 were on diuretics; 3 had received the NSAID while on ACE inhibitor and 3 *vice versa*.

The group with deterioration in renal function had a higher mean age (83.3, range 78–88) than the group with stable renal function (78.6, range 75–82). Also, more of them used diuretics (6 *versus* 2). There was no obvious difference in baseline serum creatinine. None of the 6 patients with renal deterioration had paraproteinaemia; the relevant data are shown in Table 1.

Table 1 **Clinical features of six patients with renal deterioration on combined therapy with ACE/NSAID**

Age	Risk factors	Baseline creatinine (μmol/L)	Peak creatinine (μmol/L)	Drug added	Drug stopped
81	Diuretics; malignancy	101	429	NSAID	Both
85	Diuretics; diarrhoea	58	403	NSAID	Both
88	Diuretics	58	124	NSAID	NSAID
89	Diuretics	72	157	ACEI	ACEI
79	Diuretics	95	158	ACEI	NSAID
78	Diuretics	86	146	ACEI	NSAID

ACEI=Angiotensin converting enzyme inhibitor; NSAID=nonsteroidal anti-inflammatory drug

DISCUSSION

As judged by this survey, co-prescription of an ACE inhibitor and a NSAID in the elderly commonly causes a deterioration in renal function. It could be argued that, in the 3 patients in whom an ACE inhibitor was added, unrecognized renovascular disease might have been responsible; but in 2 of these patients renal function returned to normal after stopping the NSAID (in the third patient the NSAID could not be stopped because of pain due to severe osteoarthritis). The temporal association between renal impairment with combination therapy and improvement after stopping one of the drugs indicates that the combined therapy was the cause of renal deterioration.

In a normal kidney, glomerular filtration is a function of glomerular blood flow, the balance of pressure across the capillary wall and the permeability and total surface area of the filtering capillaries. Adequate glomerular blood flow depends on cardiac output, prostaglandin-mediated afferent arteriolar vasodilatation and angiotensin II (A-II)-mediated efferent arteriolar vasoconstriction. The last two factors are especially important in states such as cardiac failure, hypovolaemia and renovascular disease, all of which are common in the elderly[3]. NSAID therapy inhibits cyclo-oxygenase, thus decreasing prostaglandin production; in consequence, afferent arteriolar flow lessens and glomerular filtration falls. ACE inhibitors depress A-II and thus inhibit A-II-mediated vasoconstriction. This lowers glomerular filtration pressure and decreases the glomerular filtration rate. A compensating reduction in systemic vascular resistance will normally raise the cardiac output and increase the renal blood flow, but elderly patients with limited myocardial reserve and volume depletion due to diuretics may not be able to increase their cardiac output sufficiently, with resultant renal hypoperfusion. The net effect of the combination therapy is a decrease in glomerular filtration rate, potentially aggravated by volume depletion and impaired myocardial function[4].

NSAIDs and ACE inhibitors are the main causes of drug-induced ARF in the elderly[5]. Since sodium depletion is a precipitating factor, addition of a diuretic increases the risk of renal failure[2]. Published reports on the renal effect of this combination are sparse. The largest series is a retrospective study of 162 patients, of whom 3 developed ARF[6]; mean age and the presence or absence of other risk factors are not recorded. In another series of 27 patients with ARF due to an ACE inhibitor, 6 were also on a NSAID[2]. There are two case reports of nephrotoxicity in elderly patients due to this combination: a 72-year-old patient who also had undetected myeloma developed postoperative ARF[17]; and a patient aged 85 developed life-threatening hyperkalaemia[8].

A limitation of our study is that the numbers are small. It is also possible that we missed some patients if the combination was prescribed during admission but was stopped before discharge because of renal deterioration.

Co-prescription of these two drug groups was rare (0.8%) and we are unable to comment on individual NSAIDs or ACE inhibitors. We do, however, conclude that this combination poses a risk of renal failure in elderly patients receiving a diuretic, who are likely to have other age-related risk factors such as poor myocardial function, vascular disease and volume depletion.

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APPENDIX B

American Journal of Kidney Diseases

The Official Journal of The National Kidney Foundation

American Journal of Kidney Diseases

The Official Journal of The National Kidney Foundation

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CONTENTS

CASE REPORTS

Enalapril-Associated Acute Renal Failure in Renal Transplants: Possible Role of Cyclosporine

Brian M. Murray, MB, BCh, MRCPI, Rocco C. Venuto, MD, Romesh Kohli, MD, and Eugene E. Cunningham, MD

● **Acute deterioration of renal function occurred shortly after the angiotensin-converting enzyme (ACE) inhibitor, enalapril, was administered to two renal transplant patients who were also receiving cyclosporine. Renal function recovered completely in both cases upon discontinuation of enalapril. Neither patient had evidence of transplant artery stenosis or chronic rejection, conditions known to predispose to renal failure during ACE inhibitor therapy. The possibility that afferent vasoconstriction induced by cyclosporine may have predisposed these patients to renal failure from enalapril is discussed.**

INDEX WORDS: Cyclosporine; angiotensin-converting enzyme inhibitor; renal failure; transplantation.

ACUTE RENAL FAILURE in renal transplants secondary to the use of angiotensin-converting enzyme (ACE) inhibitors has been described previously, but almost always in association with stenosis of the artery supplying the graft.[1-3] Two cases associated with endarteritis due to chronic rejection have also been seen.[4,5] We present two cases in which an acute deterioration of renal function occurred following administration of an ACE inhibitor, enalapril, to patients receiving cyclosporine. Discontinuation of both cyclosporine and enalapril in one case and enalapril alone in the second resulted in the return of renal function. Coexistent rejection and transplant renal artery stenosis were ruled out in both cases by renal biopsy and digital subtraction angiography, respectively. Cyclosporine can exert a toxic effect on the intrarenal vasculature. It appears initially to cause a reversible vasoconstriction that subsequently transforms into a fixed arteriolopathy affecting small preglomerular vessels.[6,7] We believe that cyclosporine-induced vasoconstriction may have predisposed these patients to the development of acute renal failure following the administration of enalapril.

CASE REPORTS

Case 1

A twenty-three-year-old white man with end-stage renal disease secondary to focal segmental glomerulosclerosis received a living related transplant on June 21, 1986. The graft initially functioned well, but 1 month posttransplant he had an episode of severe cellular rejection, which responded to therapy with OKT3. The patient's renal function stabilized, with a serum creatinine around 220 μmol/L (2.5 mg/dL) on a regimen of prednisone, 17.5 mg daily; cyclosporine, 350 mg daily; dipyramidole, 50 mg three times daily; furosemide, 60 mg daily; and atenolol, 10 mg daily. On February 17, 1987, the patient was well with a serum creatinine of 230 μmol/L (2.6 mg/dL) and a blood pressure of 170/100 mm Hg. Atenolol was discontinued and enalapril, 5 mg twice daily begun. One week later, the dose was increased to 10 mg twice daily because of persistent hypertension. On February 27, he was admitted with complaints of nausea and fatigue and found to have a serum creatinine of 830 μmol/L (9.4 mg/dL) and a blood urea nitrogen (BUN) of 64 mmol/L (169 mg/dL).

Apart from a blood pressure of 160/80 and a bruit heard in the area of the transplant, physical examination was otherwise normal. Laboratory values showed hemoglobin (Hgb), 11.5 g/L; white blood cell (WBC) count, 9.5 c/μL; platelets, 205 c/μL; serum sodium, 128 mEq/L, potassium, 5 mEq/L; chloride, 95 mEq/L; bicarbonate, 16 mEq/L, glucose, 5 mmol/L (90 mg/dL); calcium, 2.5 mmol/L (10 mg/dL); phosphate, 2.5 mmol/L (7.7 mg/dL); and normal liver enzymes. A trough cyclosporine level (by whole blood radioimmunoassay [RIA]) was 220 ng/mL. Urinalysis showed a pH of 6, 3+ protein, and trace blood with an unremarkable sediment. A renal flow and scan showed good uptake, but delayed excretion, and a sonogram showed no evidence of hydronephrosis. The patient was hemodialyzed on the day after admission. Enalapril was discontinued and cyclosporine decreased to 250 mg daily. Intravenous (IV) solumedrol, 1 g, was administered for 3 days pending the results of a renal biopsy performed on the day of admission. The biopsy showed total sclerosis of one of nine glomeruli, with a mild increase in mesangial matrix in the others. There was no evidence of rejection.

Arterial vessels were normal. Steroids were rapidly tapered and the patient's subsequent course is shown in Fig 1A. On the fourth day after admission, his urine output increased markedly from less than 1 L to over 2 L per day and his serum creatinine began to decrease spontaneously, returning to 310 μmol/L (3.5 mg/dL) by day 10. A selective renal angiogram performed the following day (Fig 2A) showed no evidence of renal artery stenosis.

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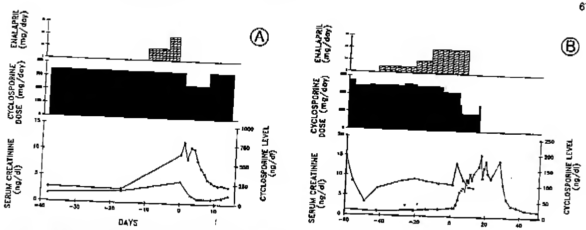
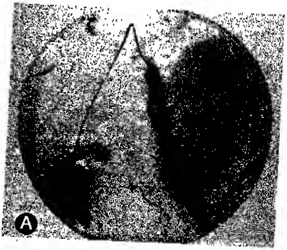


Fig 1. Sequential values of serum creatinine (●) and trough cyclosporine (▲) levels, enalapril and cyclosporine doses for (A) case 1 and (B) case 2.

Case 2

A 32-year-old woman with end-stage renal disease secondary to adult polycystic kidney disease received a cadaver transplant on August 21, 1987. Immunosuppression consisted of methylprednisolone, cyclosporine, and azathioprine. The graft functioned immediately and she was discharged 19 days posttransplantation with a serum creatinine of 106 μmol/L (1.2 mg/dL). On September 23, 1987, the patient presented with a macular rash. Serum creatinine was 97 μmol/L (1.1 mg/dL). Atenolol was stopped and enalapril, 5 mg daily, started. The rash disappeared. Over the next 6 weeks, there was a gradual deterioration in renal function, with an increase in serum creatinine to 177 μmol/L (2.0 mg/dL) despite tapering of the cyclosporine dosage from 520 to 440 mg/d. Trough cyclosporine levels (whole blood high-performance liquid chromatography [HPLC]) remained in the range of 80 to 120 ng/mL throughout.

On November 10, 1987, the patient was admitted for further investigation of her deteriorating renal function. Physical ex-



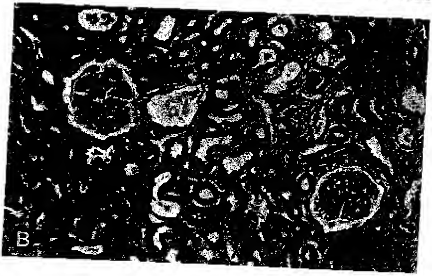


Fig 2. Selective renal angiogram of the renal transplant artery of (A) case 1 and renal biopsy (H&E, original magnification × 400) of (B) case 2.

amination showed a blood pressure of 130/90 mm Hg and was otherwise normal. Medications at the time of admission included methylprednisolone, 18 mg orally daily; cyclosporine, 440 mg daily; azathioprine, 50 mg daily; furosemide, 60 mg daily; ferrous sulfate, 325 mg three times daily; enalapril, 10 mg twice daily, which had been increased progressively in the 3 weeks before admission (Fig 1B). Laboratory values showed hematocrit 29.7; WBC count, 5.9 c/μL; platelets, 252 c/μL; sodium, 141 mEq/L; potassium, 4.3 mEq/L; chloride, 102 mEq/L; bicarbonate, 22 mEq/L; glucose, 4.4 mol/L (80 mg/dL); BUN, 22 mmol/L (58 mg/dL); creatinine, 194 μmol/L (2.2 mg/dL); calcium, 2.7 mmol/L (10.8 mg/dL); phosphate, 0.97 mmol/L (3.0 mg/dL); magnesium, 0.62 mmol/L (1.50 mg/dL); albumin, 3.6 g/dL; and normal liver enzymes. Urinalysis showed a pH of 6.0 and was negative for blood and protein with 2 to 5 WBC per high-power field, no red blood cells, renal tubular cells, or casts. Trough cyclosporine level drawn the day after admission was 101 ng/mL. Urine culture was negative. Sonography of the transplanted kidney showed mild distention of the calyceal system. A renal flow and scan showed good flow to the graft with mild hydronephrosis. A renal biopsy was performed on the third hospital day, by which time the creatinine had increased to 265 μmol/L (3.0 mg/dL). Pulse solumedrol (750 mg for 3 days) was begun pending the biopsy results and the other medications continued unchanged. Renal function continued to deteriorate, increasing to a value of 583 μmol/L (6.6 mg/dL) on the sixth hospital day. The renal biopsy (Fig 2B) showed no evidence of either vascular or cellular rejection, although there was evidence of expansion of the interstitium due to both edema and fibrous tissue, without cellular infiltration. Enalapril was discontinued and the cyclosporine dose reduced to 200 mg daily. The possibility of obstruction was ruled out by a retrograde pyelogram, which showed good drainage of contrast into the bladder. On the 15th hospital day, with no evidence of return of renal function, transplant renal biopsy was repeated and was essentially unchanged. Specifically, there was no evidence of arteriolopathy. The patient was discharged on methylprednisolone, 20 mg daily; azathioprine, 100 mg daily; nifedipine, 10 mg daily; and furosemide, 40 mg daily. Dialysis was discontinued when the serum creatinine began to decrease spontaneously, finally reaching a value of 124 μmol/L (1.4 mg/dL) on December 30, 1987. A digital subtraction angiogram performed following the recovery of renal function, showed no evidence of transplant artery stenosis.

DISCUSSION

This report describes two cases in which an acute deterioration in renal function occurred in stable functioning renal transplants, temporally related to the administration of enalapril. Although both patients received 3 days of pulse steroid therapy, the absence of histological changes suggestive of rejection on the biopsy make it unlikely that the subsequent return in renal function was due to the antirejection therapy. Neither patient had any history of hypotensive episodes, and the only potentially nephrotoxic agents they were exposed to were cyclosporine and enalapril. When reversible renal disease has occurred during ACE inhibition, it has generally been in a setting of renal hypoperfusion whether the result of renal artery stenosis,[1-3] chronic rejection,[4,5] or congestive heart failure.[6] Diuretic therapy appears to accentuate this tendency,[3] and it is of interest that both patients were receiving therapy with furosemide. However, none of the predisposing conditions mentioned here were present in either of these patients.

Another possibility is that both cases of acute renal failure were due entirely to cyclosporine. The rapidity with which renal function deteriorated was somewhat atypical for cyclosporine nephrotoxicity.[6] In case 1, only enalapril was discontinued, with prompt return of renal function several days later. While cyclosporine dosage was reduced by approximately one-third, it was subsequently increased back to the original dosage without any slowing of recovery of renal function. In case 2, both cyclosporine and enalapril were discontinued, making it difficult to decide which was primarily at fault. However, it is of interest that cyclosporine has subsequently been reintroduced in this patient (in doses up to 240 mg daily) without any deleterious effect on renal function.

In both cases there was a long delay in the recovery of renal function (10 and 30 days, respectively) after discontinuing enalapril. This was also a feature of the original report of captopril-induced renal insufficiency in transplant artery stenosis.[1] Two factors may contribute to this delay. The first is accumulation of the active metabolite of enalapril, enalaprilat, in renal failure,[9] although more likely is the induction of an actual element of acute tubular necrosis from the severity of the ischemic insult. The occurrence of renal dysfunction in both cases was temporally related to the institution of enalapril in patients whose renal function and cyclosporine blood levels had been stable on fixed doses of cyclosporine for some time before addition of enalapril (see Fig 1). This implies that enalapril was the probable cause of the sudden deterioration in renal function. Definitive proof would have required rechallenging both patients with enalapril or another ACE inhibitor. Understandably, neither patient was willing to do so.

Renal vasoconstriction induced by cyclosporine may have predisposed these patients to acute renal failure due to ACE inhibition. Hall et al[10] have suggested that intrarenal generation of angiotensin II may be important in the maintenance of glomerular filtration rate (GFR) during decreases in

renal perfusion by causing constriction of the efferent arteriole. The nephrotoxic effects of cyclosporine may be mediated at least in part by its effects on the renal vasculature.[7] The renal vasoconstrictor effects of cyclosporine were first described in the rat,[11] but there is also evidence that reversible renal vasoconstriction occurs in human renal transplants,[12] and that ultimately this may result in a fixed arteriolopathy with associated ischemic fibrosis.[7] Neither the mediators nor the intrarenal site of cyclosporine-induced vasoconstriction have been completely delineated. Both, English et al[13] and Thomson et al[14] have produced evidence that chronic cyclosporine administration in the rat induced afferent arteriolar vasoconstriction. It is of great interest that the fixed arteriolar lesions seen in chronic nephropathy are generally considered to occur on the afferent side of the glomerulus.[7] If cyclosporine does indeed result in afferent vasoconstriction and glomerular hypoperfusion, then maintenance of GFR may become dependent on generation of intrarenal angiotensin II, and inhibition of converting enzyme could result in acute deterioration of renal function. Such a decrease in GFR was recently reported by Curtis et al[15] in cyclosporine-treated renal transplant recipients following short-term ACE inhibition (for 2 days) with captopril. The exact prevalence of the phenomenon described here is difficult to establish, since there is little published experience of the effect of longer term administration of ACE inhibitors to cyclosporine-treated renal transplant recipients. We reviewed the charts of 67 patients transplanted at SUNY Buffalo in the years 1986 and 1987. Only four patients (including the two cases reported here) were treated with ACE inhibitors (enalapril in all cases) at any point in their course. The other two patients appeared to tolerate the drug well, although in both of these cases the dosage of enalapril administered never exceeded 10 mg per day.

The two cases reported here suggest that patients receiving cyclosporine may be prone to renal functional deterioration with the use of ACE inhibitors, possibly on the basis of cyclosporine-induced renal vasoconstriction. Therefore, the "captopril test" for transplant artery stenosis[1] may be less specific than in patients not receiving cyclosporine. ACE inhibitors should be used with caution in patients receiving cyclosporine and further studies are needed to address the interaction of cyclosporine with ACE inhibitors and other antihypertensive agents, particularly with respect to the effects on long-term graft function.

ACKNOWLEDGMENT

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APPENDIX C

Cardiovascular Drugs and Therapy 1:29-38, 1987

ANGIOTENSIN I CONVERTING ENZYME INHIBITORS AND THE RENAL EXCRETION OF URATE

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SUMMARY. Hyperuricaemia carries with it a high risk of tophi development affecting connective tissue in kidney, skin and joints, its overt clinical expression being gout. Diuretics, which are invariably prescribed in congestive heart failure and widely used for the treatment of essential hypertension, may cause hyperuricaemia and predispose to gout by inducing renal retention of urate.

The angiotensin I converting enzyme inhibitors captopril and enalapril have been found to augment renal urate excretion both in normal volunteers and in hypertensive patients. Current evidence appears to indicate that the uricosuric effect of captopril and enalapril could be due to the rises in renin and angiotensin I these drugs elicit by angiotensin I converting enzyme inhibition, and/or to pharmacological actions not related, at least directly, to the renin-angiotensin-aldosterone system. Serum urate levels have been significantly reduced by monotherapy with captopril in hypertensive patients suffering from hyperuricaemia. Diuretic-induced hyperuricaemia in hypertensive patients can be prevented or counteracted by the administration of captopril and enalapril.

Available clinical data support the argument that captopril and enalapril should be used as first choice drugs for the treatment of hyperuricaemic hypertensive patients. When diuretic-induced hyperuricaemia develops in patients suffering from congestive heart failure, captopril or enalapril should be added to the therapeutic regime in doses capable of countering the shift in plasma urate concentration, provided the clinical condition of the patients permits such additional pharmacological treatment.

Therapy with captopril and enalapril should preferably be instituted in a gradual manner, especially in patients with hyperuricaemia, in order to prevent the precipitation of urate in the kidney and in the urinary tract.

KEY WORDS: angiotensin I converting enzyme inhibitors, captopril, diuretics, enalapril, gout, hyperuricaemia, uricosuria

Recent investigations have disclosed that captopril is effective when used as monotherapy both in normal- and low-renin essential hypertension. In addition to angiotensin I converting enzyme (ACE) inhibition, captopril appears to lower total systemic vascular resistance by mechanisms that may be indirectly related to or independent of this direct effect; such mechanisms could include interference with the sympathetic nervous system [1,2], specific inhibition of angiotensin II generation in the kidney and other tissues [3], an increase in renal and circulating levels of kinins [4], an increase in renal and systemic production of vasodilatory prostaglandins [5,6] and an augmentation of urinary sodium output [3,7,8,9].

No ancillary properties of ACE inhibitors merited specific clinical application, except for the use of captopril in idiopathic oedema, because no incidental findings could be rationally applied, until it was noticed that captopril partially counteracted hydrochlorothiazide-induced hyperuricaemia in hypertensive patients receiving both drugs [10].

Hyperuricaemia as a Clinical Entity

The possibility that hyperuricaemia could be a risk factor for ischaemic heart disease has been disproved by recent investigations. What made hyperuricaemia an apparent risk factor was colinearity between serum urate concentration and blood pressure levels that arose within multivariate studies [11]. The clinical relevance of hyperuricaemia relates to its potential deleterious effects on the kidney and to the development of gout. Plasma urate levels above 0.42 mmol/litre (7 mg/dL) entail the risk of urate precipitation in joints, kidneys or subcutaneous tissue [11], and clinically overt gout becomes likely when serum urate concentration surpasses 0.60 mmol/litre (10 mg/dL) [12]. The incidences of hypertension, hyperuricaemia and gout are linearly related [11]; given that the most widely prescribed antihypertensive drugs, diuretics [13-24] and beta-adrenergic blockers [14,25], cause

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renal retention of urate and may thus elevate plasma urate levels, due attention must be paid to this variable in hypertensive patients. This concern also applies to congestive heart failure, in which diuretic treatment is mandatory.

Diuretics and the renal handling of urate

Within the kidney, filtered urate is handled by the proximal convoluted tubule where there is a longitudinal sequence of reabsorption, secretion and post-secretory reabsorption, or coextensive reabsorption and secretion along the segment involved; available models for the handling of urate by the proximal convoluted tubule do not discriminate between these two possible systems [15]. In man, plasma volume expansion increases and plasma volume contraction reduces the urinary fractional excretion of urate. When a diuretic is given subsequent contraction of the vascular space augments the reabsorption rates of sodium and various solutes, urate included, in the proximal tubule; in addition, the proximal tubular secretion rate of urate could become concomitantly depressed.

Diuretic-induced hyperuricaemia

The prevalence of diuretic-related hyperuricaemia is high. In a survey of 3,192 elderly hospital outpatients it was found that diuretics were used by 40% of females and 29% of males [16]. One third of these patients were hypertensive, 81% used diuretics daily and more than 70% had taken these drugs for at least 1 year. When the mean serum urate concentration of the patients taking diuretics was compared with that of the 1,405 who were not, the variable was found significantly raised both in the diuretic-treated patients as a single group and in subsets identified by the particular diuretic prescribed, including hydrochlorothiazide, chlorthalidone, furosemide, triamterene and combinations of hydrochlorothiazide and triamterene and of hydrochlorothiazide and spironolactone. These findings are at variance with the statement that potassium-retaining diuretics do not elevate plasma urate [17].

The causative relationship between diuretic therapy and hyperuricaemia in hypertensive patients is well proven and clinically relevant. In a double-blind study in which furosemide 40 mg twice-daily or hydrochlorothiazide 50 mg twice-daily were used as monotherapy in 23 and 27 patients, respectively, both groups were found to have significantly raised serum urate concentrations after the third and twelfth months of treatment [18]. In another investigation [13], 67% of thiazide-treated cases developed hyperuricaemia and 10% developed clinical gout. The early distal tubular diuretic frenquizone, significantly augmented plasma urate in 40 hypertensive patients who were treated with 10 mg daily for 1 year; the elevation was present from the fourth month of therapy [19]. In a double-blind study, hypertensive patients were subject to crossover monotherapy with chlorthalidone 25 mg, chlorthalidone 50 mg, chlorothiazide 500 mg, and chlorothiazide 1000 mg daily, for 5 weeks each; all these therapeutic regimes significantly elevated plasma urate concentrations [20]. Thus, although diuretics cause renal urate retention from the first dose [21], significant hyperuricaemia does not appear to develop in hypertensive patients until 2 to 4 months after the initiation of treatment.

Patients with congestive heart failure also tend to develop high plasma urate levels in response to diuretics [22–24]. Patients with congestive cardiac insufficiency have a reduced renal blood flow, often receive rather high doses of diuretics, and frequently have diuretic-induced hyponatraemia or suffer from plasma-volume reduction; all these facts make these patients particularly vulnerable to the development of diuretic-induced hyperuricaemia and gout. In a study involving 10 patients with congestive heart failure treated with furosemide 80 mg daily for 1 month, hyperuricaemia developed in 5 cases and overt gout in 1 case [23]. Hyperuricaemia and gout may thus develop soon after the initiation of diuretic therapy in congestive heart failure [24].

Beta-Adrenergic Receptor Blockers and Hyperuricaemia

Beta-adrenergic blockers decrease the renal excretion of urate, irrespective of their relative effects on different sub-types of receptors. A random survey of 138 published studies on the antihypertensive effects of beta-blockers has disclosed that no reference is made to serum urate in 97% of reports (Reyes and Leary, unpublished). In an open investigation in which 72 patients received atenolol 50 to 100 mg daily, serum urate rose in a statistically significant manner from a mean concentration of 0.31 to 0.34 mmol/litre [25].

Unlike diuretics, beta-adrenergic blockers do not invariably increase the mean plasma urate concentration in hypertensive patients. In individual patients the potential of beta-adrenergic blockers for inducing gout is much lower than that of diuretics. However, beta-adrenergic blocking agents increase the possibility that hypertensive patients develop gout, and when added to established diuretic therapy they may in-

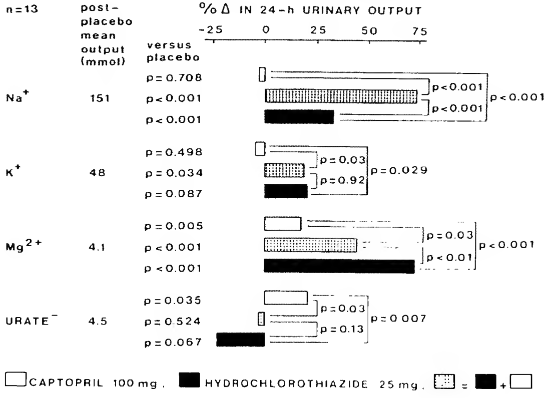
crease plasma urate concentration. This is exemplified by a study in which the mean plasma concentration of urate in 137 hypertensive patients rose in a statistically significant manner, from 0.35 to 0.37 mmol/litre, when propranolol was added to ongoing therapy with a thiazide diuretic [26].

Angiotensin I Converting Enzyme (ACE) Inhibitors Increase the Renal Excretion of Urate in Healthy Volunteers

Thirteen healthy volunteers confined to a metabolic unit were given single doses of placebo, captopril 100 mg, hydrochlorothiazide 25 mg, and a combination of the two drugs in double-blind manner and random order [21]. Urine voided after medication was collected between 0 to 3, 3 to 6, 6 to 9, 9 to 18, and 12 to 24 hours. Figure 1 depicts the excretions of sodium, potassium, magnesium, and urate that took place. There was a clear tendency for hydrochlorothiazide to cause urate retention and for captopril to augment 24-hour urinary urate output; the combination had no effect on this variable. Captopril had no natriuretic effect per se, but significantly augmented that of hydrochlorothiazide (see Figure 1). The ACE inhibitor also significantly reduced the magnesiuretic effect of the diuretic. Since these two actions of captopril could be due to ACE inhibition, and a subsequent fall in plasma aldosterone, the effect of captopril on renal urate output might be primarily ascribed to a similar mechanism.

In a study in which an analogous experimental design was followed [14], the beta-adrenergic blocking agent pindolol 10 mg, the diuretic clopamide 5 mg and a combination of these drugs were administered to 9 healthy volunteers (Figure 2). Pindolol significantly blunted, and to an extent reversed, the kaliuretic and magnesiuretic effects of clopamide, while all three active formulations decreased the renal output of urate. Even though pindolol per se exhibits a strong intrinsic sympathomimetic activity, it clearly acts as a beta-

Fig. 1. Results of an acute study carried out in healthy volunteers. Single doses of placebo, of captopril 100 mg, of hydrochlorothiazide 25 mg and of a combination of the drugs were administered separately, in random order and double-blind. Urine was collected for 24 hours after dosing. The p values depicted were derived from crossed paired t tests; the authors have set the level of significance at p < 0.05 since theoretical considerations (A.J. Reyes, unpublished), stemming from the study experimental circumstances, led to the conclusion that the risk of obtaining false positive significances was not elevated by the multiple contrasts. n = number of subjects. Adapted from [21] by courtesy of Journal of Cardiovascular Pharmacology.



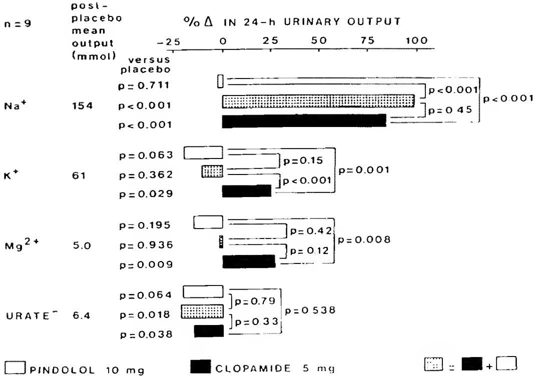


Fig. 2. Results of an acute study carried out in healthy volunteers. Single doses of placebo, of the beta-adrenergic blocker pindolol 10 mg, of the diuretic clopamide 5 mg, and of a combination of the two drugs were administered separately, in random order and double-blind. Urine was collected for 24 hours after dosing. The p values depicted were derived from crossed paired t tests; the authors have set the level of significance at $p < 0.05$ since theoretical considerations (A.J. Reyes, unpublished), stemming from the study experimental circumstances, led to the conclusion that the risk of obtaining false positive significances was not elevated by the multiple contrasts. n = number of subjects. Adapted from [14] by courtesy of Magnesium-Bulletin.

adrenergic blocker affecting the two main receptor subtypes when sympathetic drive is increased by a manoeuvre such as the administration of a diuretic [14]. The fall in the urinary outputs of potassium and magnesium caused by pindolol may be ascribed to a decrease in the activity of the renin-angiotensin-aldosterone system induced via a reduction in renal renin secretion caused by the beta-adrenergic blocker. It is noteworthy that the uricosuric effect of captopril and the urate retention provoked by pindolol were not accompanied by significant changes in mean urinary sodium output indicating that the effects of these drugs upon the excretion of urate occur independently of changes in plasma volume.

The directionally opposite effects of captopril and pindolol on renal urate excretion could depend on the opposite actions exerted by the ACE inhibitor and the adrenergic blocker on renin and angiotensin I production, or these opposite effects could be due to pharmacological properties unrelated to the renin-angiotensin-aldosterone system or to a combination of these mechanisms.

The urinary flow of urate [21] over the 24-hour period following dosage with captopril and hydrochlorothaizide is shown in Figure 3. The flow after hydrochlorothiazide 25 mg remained below that after placebo for the entire 24-hour period; the post-captopril flow exceeded that after placebo throughout the same period. This action of captopril lasted 3 to 4 times longer than the known duration of its inhibitory effect upon ACE [9,28], whereas the urate retention provoked by hydrochlorothiazide proceeded in accordance with the known duration of its renal excretory actions [21,29]. The combination of captopril 100 mg and hydrochlorothiazide 25 mg caused an increased urinary urate flow with respect to placebo during the first 8 hours after drug administration; during this period the uricosuric effect of captopril prevailed over the urate-retaining effect of the diuretic, but a slight retention of urate was caused by the combination 8 to 24 hours after dosing (see Figure 3). This retention might have been caused by the striking concomitant

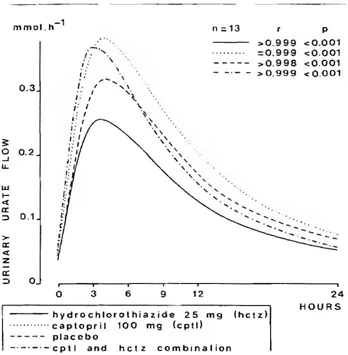


Fig. 3. Results from an acute study carried out in healthy volunteers. Single doses of placebo, of captopril 100 mg, of hydrochlorothiazide 25 mg, and of a combination of the drugs were administered separately, in random order and double-blind at hour 0 of the experiments (0800 h). Urine voided during the periods indicated in the abscissae was collected and analysed. The accumulated urate excretion at the end of each fractional collection period as a function of time was fitted by a mathematical model [27]; the mean flows shown are the derivative of those functions with respect to time. The values of the coefficients of correlation and of their significances with respect to 0 were derived from the linear transformations of the data and therefore evaluate the accuracy with which the fitted functions [21] describe the time-course of the urinary urate excretions. n = number of subjects. Adapted from [21] by courtesy of Journal of Cardiovascular Pharmacology.

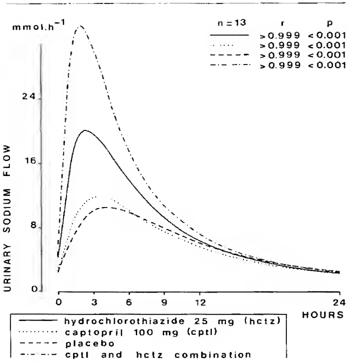


Fig. 4. Results of an acute study carried out in healthy volunteers. Single doses of placebo, of captopril 100 mg, of hydrochlorothiazide 25 mg, and of a combination of the two drugs were administered separately, in random order and double-blind at hour 0 of the experiments (0800 h). Urine voided during the periods indicated in the abscissae was collected and analysed. The accumulated sodium excretion at the end of each fractional collection period as a function of time was fitted by a mathematical model [27]; the mean flows shown are the derivatives of those functions with respect to time. The values of the coefficients of correlation and of their significances with respect to 0 were derived from the linear transfarmations of the data and therefore evaluate the accuracy with which the fitted functions [21] describe the time-course of the urinary urate excretions. n = number of subjects. Adapted from [21] by courtesy of Journal of Cardiovascular Pharmacology.

natriuretic (Figure 4) and diuretic effect of the combination which occurred during the first 6 hours after dosing, which presumably diminished the intravascular volume to such an extent that proximal tubular urate reabsorption was markedly increased at a time when the urate-retaining effect of hydrochlorothiazide predominated over the fading uricosuric effect of captopril (see Figure 3).

To gain insight into the renal tubular effects of captopril that account for the uricosuric action of the substance, it would first be necessary to reduce serum urate with a non-uricosuric drug, and to observe whether the uricosuric effect of captopril persisted; if such were the case, an increase in tubular secretion of uric acid would not be the sole mechanism responsible for captopril-induced uricosuria. Pyrazinamide blocks the proximal tubular secretion of uric acid [13]; therefore if this substance blunted the uricosuric effect of captopril, a diminution of urate reabsorption could partly account for captopril-induced uricosuria. Captopril is excreted by the kidney both as untransformed captopril and as metabolites [28]. In normal volunteers, 78% of the renal excretion of the parent drug is effected by secretion at the proximal tubule, a finding consistent with the fact that coadministration of probenecid increases plasma captopril concentration and decreases its renal disposal by causing an approximate 50% reduction in proximal tubular secretion [30]. A direct proximal tubular action of captopril could pos-

sibly account for its effect upon the renal handling of urate.

The uricosiuric property of ACE inhibitors is shared by enalapril. In a study in which 10 healthy volunteers were given enalapril 10 mg daily, the renal fractional excretion of urate was increased during the 4 to 8 hour interval after dosing on the eighth day [31]. This was accompanied by a parallel increase in the fractional excretion of phosphate which was also provoked by captopril. This interference with the renal handling of phosphate by enalapril points to a specific effect of this substance upon the transport mechanisms of the proximal convoluted tubule.

Angiotensin I Converting Enzyme Inhibitors and Uricaemia in the Clinical Setting

Hypertensive patients with normal serum urate levels

A multicentric study lasting 6 to 52 weeks involved 255 patients with mild to moderate essential hypertension who were randomly assigned to treatment with captopril, hydrochlorothiazide or a combination of these drugs [10]. The administration of 25 mg captopril thrice-daily, in a subgroup of 69 cases, caused a statistically nonsignificant fall in serum urate from 0.33 to 0.32 mmol/litre; the administration of hydrochlorothiazide 15 mg thrice-daily, in 67 cases, significantly increased plasma urate levels from 0.33 to 0.44 mmol/litre; combined therapy with both drugs in the doses quoted significantly raised serum urate concentration from 0.34 to 0.40 mmol/litre. While no significant differences were detected between any mean pretreatment plasma urate values, final mean plasma urate concentrations differed in a statistically significant manner between the captopril- and hydrochlorothiazide-treated groups, between the groups that received captopril and the combination therapy and between the hydrochlorothiazide- and the combination-treated groups. These results appear to indicate that captopril partially counteracts the hyperuricaemic effect of hydrochlorothiazide, although the doses in which the drugs were given would not have blunted the latter action completely.

In another multicentric study [26], hypertensive patients not controlled by previous diuretic therapy received added captopril or propranolol for 12 weeks. In the 133 patients given captopril and a thiazide, plasma urate concentration changed nonsignificantly from a mean value of 0.35 to 0.36 mmol/litre.

The effects of enalapril 20 mg daily, hydrochlorothiazide 50 mg daily and of their combination were studied in 190, 188 and 94 hypertensive patients, respectively, in a randomised, double-blind, parallel multicentric investigation that lasted for 8 weeks [32]. Dosage was doubled when supine diastolic blood pressure was above 90 mm Hg at the end of the fourth week of treatment. Comparisons between serum urate concentrations before treatment and at the end of the eighth week yielded no significant difference between mean values for enalapril (0.38 mmol/litre in both instances), but disclosed a significant rise after hydrochlorothiazide (0.37 to 0.46 mmol/litre). Patients treated with combination therapy had a change in mean serum urate concentration from 0.38 to 0.44 mmol/litre, the statistical significance of which is not quoted by the authors; however, the post-treatment mean value was significantly lower than that after hydrochlorothiazide.

In a randomised double-blind study, 22 hypertensive patients were treated with captopril 50 to 100 mg and hydrochlorothiazide 50 mg daily for a 4-week period; mean serum urate concentration changed from 0.29 to 0.33 mmol/litre [33]. Twenty one of these patients later received captopril 50 to 100 mg and hydrochlorothiazide 25 mg daily; their mean serum urate concentration was 0.30 mmol/litre after approximately 8 months of this regimen. Thereafter 17 of the same group of patients were treated with captopril 50 mg and hydrochlorothiazide 50 mg daily for a further month; mean serum urate concentration fell to 0.29 mmol/litre. None of these changes of mean plasma urate levels differed significantly from the corresponding values before treatment was initiated.

Enalapril 10 to 40 mg daily or a combination of enalapril in the same dose range with hydrochlorothiazide 50 mg was administered to hypertensive patients for 1 year [34]. Plasma urate concentration was significantly reduced in the enalapril-treated group from a mean pre-treatment value of 0.33 to 0.28 mmol/litre at the end of therapy. In the group of 16 patients who received combination therapy serum urate changed in a statistically insignificant manner from 0.30 to 0.33 mmol/litre in the same period. Some of the patients who participated in this investigation were also studied to determine their renal clearance of urate, normalised for glomerular filtration rate. The renal clearance of urate increased with respect to corresponding individual post-placebo values in 14 cases treated with enalapril and remained unchanged in 3 cases.

Seventeen hypertensive patients received captopril 50 mg twice daily as monotherapy in an open study lasting 3 months [35]. Serum urate concentration decreased significantly from 0.43 to 0.39 mmol/litre.

The studies in normouricaemic hypertensive pati-

ents described above allow the conclusion that both captopril and enalapril reduce plasma urate concentration, provided the dose administered is adequate and therapy is sufficiently prolonged for the effect to attain statistical significance. In addition, both ACE inhibitors may totally counteract the increases in plasma urate concentration that diuretics induce in normouricaemic patients, provided the dose relationship between the drugs administered is appropriate.

Hypertensive patients with hyperuricaemia

In an open study carried out in two centers, captopril 100 mg was given as daily monotherapy to 20 hypertensive patients with hyperuricaemia [36]. Ten patients (group A) maintained their usual low-sodium (60 to 120 mmol daily), low-purine diet throughout the study, whereas the 10 other cases (group B) continued their habitual diet, which was relatively rich in sodium (180 to 200 mmol daily) and purines. Supine and erect blood pressures were measured and venous blood samples were drawn for analysis of serum urate levels before active therapy and 20 to 22 hours after the last dose of captopril. After 4 weeks of treatment, mean serum urate values fell from 0.46 to 0.35 mmol/litre (24% reduction) in group A and from 0.53 to 0.46 mmol/litre (13% reduction) in group B (Figure 5). The mean fall in serum urate concentration recorded in group A patients was significantly greater than that in group B. It is common experience in clinical pharmacology that the effect of any therapy is greater the wider the deviation of the pre-treatment value from its normal range. The fact that this type of response did not occur in the study under consideration, would primarily indicate that differences in diet or changes in blood pressure responses were involved.

It is unlikely that differences in purine intake could account for the dissimilar responses observed, since the diets taken by the patients in both groups during the study had been followed for months prior to the initiation of treatment with captopril, ACE inhibitors have no known effects upon purine metabolism per se, and the uricosuric effect of captopril would suffice to explain the change in uricaemia observed. It is likely that differences in sodium intake might have influenced urate excretion by affecting plasma volume; if such had been the case group A would have responded to therapy with lower uricosuria, following enhanced reabsorption of sodium in the proximal tubule, and a subsequently lower fall in mean serum urate than group B. The greater response of plasma urate levels in group A could have been related to the effects of captopril on renin and/or on angiotensin I, since the activity of the renin-angiotensin-aldosterone system must have been relatively enhanced by the low-sodium diet in this group. In addition the low-sodium diet in group A could have indirectly potentiated the hypouricaemic action of captopril, since monotherapy of hypertension with once-daily captopril exerts a significant antihypertensive effect that lasts for nearly 24 hours after dosing when patients are on a sodium-restricted diet [37], which was the case in group A but not in group B (Figure 6). Inasmuch as arterial blood pressure and plasma urate concentration are fairly well related in increasing linear fashion, the greater drop in mean plasma urate concentration in group A could be at least partly explained by the clinically significant and sustained fall in blood pressure induced by once-daily captopril in group A, provided an actual mechanism existed for a causative relationship between the haemodynamic and biochemical variables.

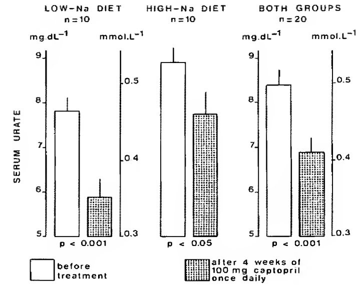


Fig. 5. Results (mean ± SEM) of a bi-centric open study. Hyperuricaemic hypertensive patients were treated with captopril 100 mg once daily as a monotherapy for 4 weeks. Patients in the low-sodium diet group (60 to 120 mmol daily) also followed their usual purine-restricted regime, whereas patients in the high-sodium diet group (180 to 200 mmol daily) also took their habitual high-purine diet. Plasma urate concentration was assessed by a colorimetric reaction using a Wiener kit in the low-sodium diet group and by an enzymatic colorimetric method using a Boehringer-Mannheim Peridochrome kit in the high-sodium group. Post-treatment assays were carried out 20 to 22 hours after the last intake of captopril. n = number of subjects. Adapted from [36] by courtesy of South African Medical Journal.

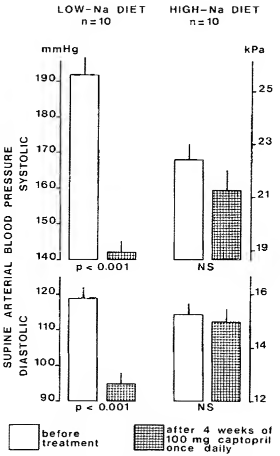


Fig. 6. Results (mean ± SEM) of a bicentric open study. Hyperuricaemic hypertensive patients were treated with captopril 100 mg once daily as a monotherapy for 4 weeks. Patients in the low-sodium diet group took their usual 60 to 120 mmol of the cation daily, whereas patients in the high-sodium diet group followed their habitual intake of 180 to 200 mmol per day. Post-treatment blood pressure measurements were carried out 20 to 22 hours after the last intake of captopril. n = number of subjects. Adapted from [36] by courtesy of South African Medical Journal.

Clinical Application of the Uricosuric and Hypouricaemic Effects of Captopril and Enalapril

In hypertensive patients with hyperuricaemia, ACE inhibitors should undoubtedly be considered as possible first-choice drugs, in accordance with the tendency to individualize the treatment of high blood pressure that is replacing the rigid norm set by the stepped-care approach. The fact that both captopril and enalapril can counteract diuretic-induced hyperuricaemia makes the coadministration of ACE inhibitors and diuretics appropriate.

The clinical administration of captopril and enalapril in patients with hyperuricaemia, whether of endogenous or iatrogenic origin, requires the same precautions applied when any other uricosuric agent is used in these patients. An acute increase in urinary urate excretion may give rise to the precipitation of urate within the renal tubules or in the urinary tract distal to them [11,13]. Renal insufficiency may follow these alterations by tubular and/or renal tract obstruction. Macroscopic urinary stones and renal colic are also possible complications in these circumstances. These events are more likely in patients with a history of urate renal calculi or clinical gout [11]. The addition of an ACE inhibitor to ongoing diuretic therapy may precipitate copious uricosuria due to pre-existing urate retention provoked by the diuretic; this would render urate precipitation likely [11], although increased diuresis might reduce the possibility of such an event [38]. Renal complications of increased urate excretion may be prevented or attenuated by prescription of a fluid intake of at least 2 litres daily thereby diluting the urine and reducing the chances of urate precipitation [13]. Alkalinization of urine during the first 3 days of therapy with ACE inhibitors would reduce the likelihood of urate precipitation by increasing its solubility [13]. However urine should not be alkalinized in patients with a history of renal stones of unknown chemical composition, since only urate precipitation would be prevented, whereas bicarbonate precipitation could be promoted by alkalinization.

Since it appears that the hypouricaemic effect of captopril is dose-related in the range 25 to 100 mg/day and this effect may be solely ascribed to its uricosuric action, it would be prudent to initiate captopril therapy at low doses to avoid massive uricosuria. Whenever precipitation of uric acid within the renal system is liable to develop, renal function should be closely monitored, especially during the first 2 weeks of therapy. Evidence of loin pain or tenderness should be sought in all captopril treated cases.

The uricosuric diuretic tienilic acid [17,39] is still in current use in some countries and other uricosuric diuretics, such as indacrinone [40] and A-53385 [41], are under investigation. The interaction between ACE inhibitors and uricosuric diuretics should constitute a research priority given the common effect of both drug groups on the urate content of the urine.

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